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MECHANISM OF ACTION OF STEROID HORMONES

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PREFACE

RESEARCH in steroid endocrinology over the previous decade had been directed primarily toward outlining the metabolic pathways involved in the synthesis and catabolic transformations of the steroid hormones. More recently interest has been growing in the field of the mechanism of action of these important, growth-regulating substances and it seemed clear that the time was ripe for a general survey of our current concepts of these mechanisms. Enough information had accumulated on which to base fruitful discussions of areas deserving further exploration. It was believed that this could be achieved most effectively by a small group which would spend some three days in an intensive consideration of possible chemical loci of hormone action. Since the more physiologic aspects of hormone action have been reviewed elsewhere, it was decided to concentrate our attention primarily on the chemical and physical events occurring at the molecular level. To achieve this end, a number of organic and physical chemists, physicists, and biochemists with broad experience in enzymology were invited to participate in the meeting together with endocrinologists and steroid biochemists who have been working directly in this area. It was believed that this experience might be mutually enriching and might lead to suggestions for future work.

The Conference was made possible by a generous grant from the National Science Foundation and we wish to express our deep appreciation to them. A number of scientists who had accepted invitations were unable to attend at the last minute for reasons of health or conflicting engagements. We deeply regret that Drs. D. H. R. Barton, Carl F. Cori, Bernard Pullman and Paul Talalay were unable to attend the Conference and participate in the discussions. Our thanks are due to the speakers and contributors to discussions for their co-operation in supplying the editors with manuscripts promptly and in correcting the recorded discussion. Our thanks are also due to Mrs. Patricia Grendell for her services in preparing the manuscripts for publication and to Pergamon Press for publishing the record promptly.

September, 1960

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MECHANISMS OF ACTION OF STEROID HORMONES: STATEMENT OF THE PROBLEM*

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THE recent rapid extension of our knowledge of the chemistry, biochemistry and physiology of steroid hormones has resulted in focusing a great deal of attention upon these substances as the regulators of biological processes. Over the past ten years the principal interest in biochemistry of steroid hormones has centred upon the mechanisms for the synthesis and disposal of these substances. Although many reactions and many details still remain to be unraveled, the principal pathways of biosynthesis and degradation of these compounds now seem to be firmly established. During this period, too, we have seen the unfolding of the main outlines of the pattern of synthesis, transport and disposal of the principal cellular constituents. At the same time the metabolism of the steroid hormones has been woven into this fabric. In parallel with these biochemical and physiologic developments, there has been an enormous expansion of interest in the synthesis and biological testing of substances related to the naturally occurring steroid hormones. This activity has yielded a large number of steroids and related compounds which mimic the biological activity of the natural hormones. Indeed, in many cases the products of partial or total synthesis have been found to be more active than their natural counterparts and have therefore found their way to therapeutic use.

Thus the stage seems to be set for an inquiry into the mechanisms whereby the steroid hormones integrate and regulate the processes of growth and metabolism. This question, it seems to me, lies at the heart of one of the major problems of modern biology, the regulation of growth. Stated most generally, we may ask how is the enormously complex fabric of biochemical reactions in a living organism controlled, integrated and modified so as to meet the demands of growth, of reproduction, and of the stresses of an ever-changing environment?

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This Conference was conceived with the idea that the time is now appropriate to summarize the presently available information concerning the mechanism of action of steroid hormones, to consider possible new approaches, and to discuss the ways and means whereby the modern tools of biochemistry and biophysics may be brought to bear in a decisive fashion on this problem. The increasing interest in this field is best demonstrated by noting the increasing number of papers published which bear directly or indirectly upon mechanism of action (1–5).

The definition at the physiological level of the mechanism of action of steroid hormones is too well known to require elaboration. In its major phases it is beyond argument and dispute. It is when we turn to the definition of the mechanism of action at the molecular level that we begin to tread upon slippery and treacherous ground. Indeed, there is no agreement as to just what is meant by "mechanism of action of steroid hormones"; this phrase has different meanings for different investigators. That this is so is demonstrated by the vigor of the disagreements and controversies in this field, the difficulties of obtaining substantial evidence and the even greater difficulty at times of obtaining agreement concerning the interpretation of the evidence.

How can one, then, define the mechanism of action of steroid hormones at the molecular level? Unless we are to be engulfed by mysticism, we are forced to define a hormone as a substance which exerts chemical control over one or more metabolic reactions. Britton Chance (6) has recently discussed chemical control and given some definitions which seem particularly pertinent to our present discussion. Although his discussion revolves about carbohydrate metabolism in ascites tumor cells, the general statements seem to be pertinent to the present problem. One may define a hormone as "a chemical control mechanism in which the concentration of an essential intermediate becomes rate-limiting because its rates of formation and disappearance are unbalanced so as to favor the latter". In pursuing this line of thought, Chance then proceeds to define the desirable properties of a control substance as follows: "The first consideration is that the control substance influence a variety of systems . . . or a key point in one large system or both . . . A second criterion for a control intermediate is high affinity for the control substance whereby a large control response could be obtained with only a small build-up in the concentration of the rate-controlling intermediate. In fact, it is important that the rates of formation and utilization of the control substance be finely balanced, so that no great excess of the control substance accumulates and thereby prevents a rapid reversibility of the control. Reversibility is highly desirable in a control reaction and may be accomplished in two ways. First, the control chemical may be an active participant in the metabolic process and thereby be expended. . . . On the other hand, if the control substance is not a direct participant in the metabolic system, then chemical processes for its decomposition or physical processes for its segregation must be available in order to reverse the control effects. The type of control process that one considers for the complex sequence of reactions involved in cell metabolism is necessarily a continuous one wherein the rate of metabolism is proportional over a reasonable range to the concentration of a control substance. Higher concentrations of the control substance may fail to give any rate increase, as for example, by the saturation of an enzyme system. It is obvious that the metabolism is no longer under control of the reaction in question, on the horizontal portion of the control characteristic curve." These statements paraphrase in biochemical and kinetic terms the classical definitions of hormones and hormone actions. Our problem, then, is to devise experimental approaches whereby the locus or loci of these actions may be determined and the detailed molecular mechanisms uncovered.

Two general types of approach have been chosen for studying the mechanism of action of hormones. In the first, the analytical approach, attempts are made to study alterations in biochemical reactions in specific target tissues of experimental animals subjected to treatment with hormones. In early experiments the procedure may consist of pretreatment of the experimental animal with the hormone and then removal of a suitable tissue and comparison of its biochemical reactions with those of the unstimulated tissue. An important feature of this approach is to employ progressively shorter time intervals between hormone administration and biochemical study so as to approach as closely as possible the locus of initial action of the hormone. If differences between stimulated and unstimulated tissues are found, the next stage is to determine whether the hormone incubated *in vitro* with the target tissue will produce a similar effect. This has been the classical approach employed by Mueller (4) in the case of the estrogens, as well as by other investigators in the exploration of mechanism of action of other types of steroid hormones. This is a fruitful approach, and has the advantage that a relation between physiologic activity and biochemical activity may be kept in perspective.

The second, the inductive or synthetic approach, is based upon the establishment of a working hypothesis that the mechanism of action of a hormone may be mediated through some biochemical or biophysical phenomenon. Attempts are then made to seek out and study a system in which this hypothesis can be tested. Such an approach is fraught with great risks and may lead down many blind alleys, but has the basic advantage that one may start with a simple system which can be defined chemically and proceed to systems of greater complexity which resemble more closely those of physiological interest. Neither of these methods can be used to the exclusion of the other, and it is more than likely that information obtained by both procedures will be needed to lead us along the right path.

What types of hypotheses have been proposed for the mechanism of action of steroid hormones? The first one I should like to consider is of physiologic derivation and postulates an effect of the steroid hormone at the cell surface in its most basic form, and at intracellular phase boundaries in modified form, If steroid hormones acted only upon surfaces of intact cells, then the use of broken cell preparations for the study of steroid hormone action would be pointless. However, many suggestive effects of steroid hormones in *in vitro* systems have been obtained with such preparations. Modification of this hypothesis may also be required by the growing recognition of the importance of pinocytosis in the transfer of metabolites through the cell wall. It thus becomes necessary to alter the hypothesis to read "phase boundaries within the cell". If by association with some specific site, either on the cell surface or at the membranous envelopes of some of the cytoplasmic organelles, the steroid hormone acted so as to alter the rate at which substances crossed the phase boundary, it could serve in the role described above. Such a role for steroid hormones is appealing, since they are known to be surface active and to be highly oriented at phase boundaries. If one inquires more closely into this concept, one may ask a second question, namely, if a steroid hormone acts in this way, does it exert its effect by interacting with an enzyme system upon the cell surface or at the phase boundary, or is its action a physical and less readily definable one? Evidence upon this point is lacking, but it must be recognized that action at a phase boundary and action upon an enzyme system are by no means mutually exclusive.

This brings us to the second major hypothesis, namely, that the steroid hormones exert their effects by interacting with enzyme systems. Again, two cases may be distinguished: one, in which the steroid itself is a participant in the enzymatic reaction and serves in a role similar to that of a coenzyme, and the second, a less precisely defined role, in which the steroid interacts with an enzyme system and affects the reaction rate by altering the kinetic characteristics of the reaction. Modern tools of reaction kinetics hold the promise of shedding much light on the nature of the interactions involved.

The first hypothesis has been proposed by Talalay (7, 8) and it had a certain currency which derives from the fact that many of the steroid hormones undergo oxidative and reductive changes during the course of their metabolism. It has been an appealing hypothesis to equate these changes with a mechanism whereby they exert their effects. However, at the present time, the evidence that this is the case has not been forthcoming (9), and it is indeed a little difficult to conceive of the steroid hormones as playing an obligatory role in metabolic processes such as would be required if they functioned as coenzymes.

A second type of mechanism which is much less precisely defined proposes for the steroid hormones a role in the alteration of the rates of key metabolic reactions (5). The effects need not be large ones, as was pointed out by Chance (6), for a build-up of metabolites in either direction. The way in which the steroids exert their effects has not been delineated, although attractive working hypotheses may be set up.

A third type of approach to this problem comes from consideration and correlation of our knowledge of the chemical, physical and biological properties of steroid hormones. An understanding of these relations between chemical structure and biological activity of the steroids may be an important key to our understanding of the mechanism of their action. The enterprise of the organic chemists has made available for study many analogs and structural relatives of the naturally occurring steroid hormones. At one point it seemed almost possible to make useful generalizations concerning the relations between structure and activity. But with the increasing complexity of these analogs, the task becomes more complicated. The recent great strides in our knowledge of the structure and electronic configurations, as well as the more subtle aspects of the stereochemistry of steroids, have given us a new perspective on this problem. It is almost banal to state that a detailed knowledge of the role of the functional groups on the steroid molecule is important for our understanding of its biologic activity, for it is clear from the most cursory survey that alteration of these groups alters biological activity, although the specific effects of alterations in structure cannot yet be predicted with precision.

Our approach to this problem has in the past to a large extent been a static one, although dynamic factors have always been implicit in the analysis. It may well be that in order to understand more fully the relations between structure and biologic activity, a more dynamic approach may be necessary; one which, for example, takes into account chemical reactivity as influenced by the action of the long range effects revealed by the brilliant work of Barton (10–12). The precise role of these effects in enzymatic reactions and the interactions between enzyme–substrate binding on the one hand and intrinsic reactivity on the other may hold important clues for our understanding of the interaction of a steroid hormone with the locus at which it exerts its specific effect. For these reasons, the consideration of the interactions of steroid hormones with biologically important compounds, such as proteins, nucleotides and constituents, has been given a prominent place in this Conference.

Finally, before throwing these general remarks open for discussion, it would seem desirable to propose certain criteria to serve as frames of reference for a discussion as to what evidence we consider acceptable and relevant to mechanism of action. Since at the present time there exists a wide gap between effects of steroid hormones upon enzymatic reactions in isolated and purified systems and the physiologic effects which they exert, one must first postulate some rough parallelism between biological activity in the whole animal and the effect on the *in vitro* system. Exact parallelism cannot be expected, since the simplified system removes to a large extent the factors of transport, absorption and inactivation which are an integral part of the total mechanism of action in the whole animal. For the time being, then, a rough parallelism is the best that can be expected. An important criterion, however, is a consideration of the structural specificity for biological activity which is manifest in such striking fashion with the steroid hormones. The remarkable effects of alterations of stereochemistry at a single carbon atom upon biological activity should be guide lines for the design of *in vitro* experiments. Thus, in studying the *in vitro* effects of steroids upon enzyme systems, it is of crucial importance to study both biologically active and inactive steroids. If they exert the same effects or similar effects upon the system studied, then it seems *a priori* highly unlikely that the effect observed is physiologically significant. It is important, too, in such studies to examine physical properties and to search for parallelisms between physical properties and the biological or biochemical properties which are the main subject of investigation. Some steroid effects may be due to structural characteristics not primarily related to their biological specificity.

A second and highly important consideration is that of concentration. From the results of studies with steroid hormones labeled with tritium and having extremely high specific activities, one may obtain some notion regarding the concentration of steroid actually located in the target site. These experiments have reduced our estimate of concentration by several orders of magnitude, and it is important in our *in vitro* counterparts for steroid hormone action to use concentrations which are compatible with those that can be obtained in the whole animal. One qualification must be added here, namely, that the concentration required for biological activity in the *in vivo* system may be increased by virtue of the presence in the whole animal of mechanisms for the disposal of the hormone. By the same token, in crude tissue preparations enzymes active in the degradation of steroids may be present, and thus require the addition of excessively large amounts of steroids. For this reason it is important in *in vitro* experiments to determine whether or not the added hormone remains in its added form or has been converted to some metabolite.

In summary, I would like to offer a cliche and a corollary. The cliche is that there is nothing about the chemical or physical properties of the steroid hormones which is irrelevant to their biological activity. The corollary is that all of these properties are not equally important. It is one of the tasks of this Conference to establish criteria whereby the important and the less important may be distinguished.

REFERENCES

1. ENGEL, L. L., *Essays in Biochemistry* (edited by GRAFF, S.) pp 85–93. John Wiley, New York (1956).
2. ENGEL, L. L., *Vitamins and Hormones* (edited by THIMANN, K. V. and HARRIS, R. S.) Vol. 17, pp. 205–222. Academic Press, New York (1959).
3. HECHTER, O., *Vitamins and Hormones* (edited by THIMANN, K. V. and HARRIS, R. S.), Vol. 13, pp. 293–346. Academic Press, New York (1955).
4. MUELLER, G. C., HERRANEN, A. M. and JERVELL, K. F., *Recent Progr. in Hormone Research* **14**, 95 (1958).
5. VILLEE, C. A., *Perspectives in Biol. and Med.* **2**, 290 (1959).
6. CHANCE, B., *Amino Acids, Proteins and Cancer Biochemistry* (edited by EDSALL, J. T.) pp. 191–212. Academic Press, New York (1960).
7. TALALAY, P. and WILLIAMS-ASHMAN, H. G., *Proc. Natl. Acad. Sci.* **44**, 15 (1958).
8. TALALAY, P., HURLOCK, B. and WILLIAMS-ASHMAN, H. G., *Proc. Natl. Acad. Sci.* **44**, 862 (1958).
9. HAGERMAN, D. D. and VILLEE, C. A., *J. Biol. Chem.* **234**, 2031 (1959).
10. BARTON, D. H. R. and HEAD, A. J., *J. Chem. Soc.* 932 (1956).
11. BARTON, D. H. R., HEAD, A. J. and MAY, P. J., *J. Chem. Soc.* 935 (1957).
12. BARTON, D. H. R., MCCAPRA, F., MAY, P. J. and THUDIUM, F., *J. Chem. Soc.* 1297 (1960).